(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 March 2004 (18.03.2004)

PCT

(10) International Publication Number
WO 2004/022713 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number:
PCT/US2003/027798

(22) International Filing Date:
5 September 2003 (05.09.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/408,809 5 September 2002 (05.09.2002) US

(71) Applicant: TECHLAB, INC. [US/US]; 1861 Pratt Drive, Suite 1030, Blacksburg, VA 24060-6364 (US).

(72) Inventors: BOONE, James, Hunter; 545 Arrowhead Trail, Christiansburg, VA 24073 (US). LYERLY, David, Maxwell; 204 MacArthur Avenue, Radford, VA 24141 (US). WILKINS, Tracy, Dale; 6254 Chestnut Ridge Road, Riner, VA 24149 (US).

(74) Agents: DICKMAN, Jean, M. et al.; Shook, Hardy & Bacon L.L.P., 2555 Grand Blvd., Kansas City, MO 64108-2613 (US).

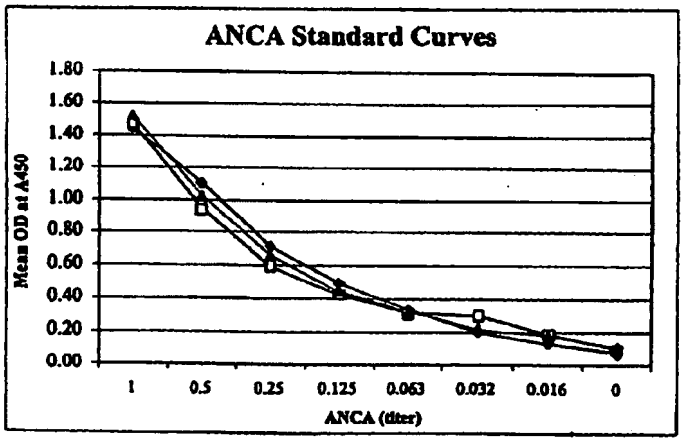
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

[Continued on next page]

(54) Title: METHOD FOR DISTINGUISHING ULCERATIVE COLITIS FROM CROHN'S DISEASE BY DETECTING THE PRESENCE OF FECAL ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODIES (ANCA)



(57) Abstract: A method and apparatus for the differentiation of ulcerative colitis from Crohn's disease and other gastrointestinal illnesses using the presence of anti-neutrophil cytoplasmic antibodies (ANCA) as a marker of ulcerative colitis is described. The apparatus consists of either a qualitative enzyme-linked immunoassay or other immunoassay that utilizes antibodies specific to human immunoglobulins for the measurement of total endogenous ANCA in a human sample. The method and apparatus can be used by healthcare providers to distinguish ulcerative colitis from Crohn's disease and other gastrointestinal.

WO 2004/022713 A2

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

METHOD FOR DISTINGUISHING ULCERATIVE COLITIS FROM CROHN'S DISEASE BY DETECTING THE PRESENCE OF FECAL ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODIES (ANCA)

BACKGROUND OF THE INVENTION

This invention relates to non-invasive methods for differentiating clinical subtypes of Inflammatory Bowel Disease, namely Crohn's disease (CD) and ulcerative colitis (UC). More specifically, this invention relates to a method and apparatus for aiding in the differentiation of Crohn's disease from ulcerative colitis by determining the presence of anti-neutrophil cytoplasmic antibodies (ANCA), wherein the presence of ANCA is indicative of ulcerative colitis. In addition, the presence of fecal ANCA may be used to differentiate ulcerative colitis from other gastrointestinal illnesses such as Irritable Bowel Syndrome.

An estimated 1 million Americans suffer from Inflammatory Bowel Disease (IBD). IBD is characterized by a chronic inflammatory response that results in histologic damage to the intestinal lining. Crohn's disease may involve the entire gastrointestinal tract and include inflammation extending into the transmural mucosa, whereas ulcerative colitis affects solely the large bowel and includes inflammation of the innermost lining. These two distinct diseases require a rapid differential diagnosis for optimal treatment. Conventional methods utilizing multiple endoscopy examinations and histological analysis may take years to confirm a diagnosis. U.S. Patent No. 6,218,120 discloses a method of determining the presence of serum ANCA as a marker to diagnose IBD. However, it does not disclose a method for diagnosing ulcerative colitis in a patient diagnosed with IBD. Further, the method does not disclose testing human feces for the presence of ANCA.

Accordingly, there remains a need in the diagnostic industry for a non-invasive method of differentially diagnosing ulcerative colitis from Crohn's disease or other gastrointestinal illnesses.

SUMMARY OF THE INVENTION

Accordingly, in one of its aspects, the present invention provides non-invasive methods for differentiating between diagnoses of ulcerative colitis and Crohn's disease.

In another of its aspects, the present invention provides methods for differentiating between ulcerative colitis and Crohn's disease wherein the presence of fecal ANCA is used as a marker for ulcerative colitis.

In a further aspect, the present invention provides immunoassays, e.g., and enzyme-linked immunoassays, that utilize antibodies specific to human immunoglobulins for the measurement of total endogenous ANCA in human feces.

In yet another of its aspects, the present invention provides methods differentially diagnosing ulcerative colitis from other gastrointestinal illnesses such as Irritable Bowel Syndrome (IBS). In still another of its aspects, the present invention provides methods for diagnosing ulcerative colitis wherein the presence of ANCA is used as a marker for ulcerative colitis.

According to the present invention, the foregoing and other aspects are achieved by a non-invasive method for aiding in the differentiation of ulcerative colitis from Crohn's disease in a patient presenting with IBD. In the method of the present invention, fecal ANCA are used as a marker and the presence of ANCA indicates a differential diagnosis of ulcerative colitis. This rapid diagnosis may then be used by healthcare professionals to prescribe proper treatment.

Aspects of the present invention are further achieved by immunoassays that utilize antibodies specific to human immunoglobulins for the measurement of total endogenous ANCA in human feces.

Additional aspects of the invention, together with the advantages and novel features appurtenant thereto, will be set forth in part in the description which follows, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned from the practice of the invention. The objects and advantages of the invention may be realized and attained by means, instrumentality's and combinations particularly pointed out in the appended claims.

BRIEF DESCRIPTION OF THE VIEW OF THE DRAWING

Fig. 1 is a graphical representation of a standard curve of anti-neutrophil cytoplasmic antibodies in accordance with an embodiment of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to non-invasive methods for differentiating between ulcerative colitis and Crohn's disease using the presence of fecal ANCA as an indicator of ulcerative colitis. The present invention also is directed to a method for differentiating between ulcerative colitis and other gastrointestinal illnesses such as IBS. The present invention is further directed to immunoassays that utilize antibodies specific to human immunoglobulins for the measurement of total endogenous ANCA in human feces. The particular embodiments described herein are intended in all respects to be illustrative rather than restrictive. Alternative embodiments will become apparent to those skilled in the art to which the present invention pertains without departing from its scope.

ANCA specific immunoassays may be used to differentiate ulcerative colitis and indeterminate colitis from Crohn's disease by measurement of the presence of total endogenous ANCA. In addition to fecal matter, a sample of whole blood, serum, plasma or other bodily fluid or tissue may be tested for ANCA to diagnose ulcerative colitis. This differential diagnosis may then be used by healthcare professionals for determining optimal treatment. A qualitative immunoassay, such as a later flow dipstick that utilizes both monoclonal and polyclonal antibodies to endogenous human ANCA to indicate the presence of ulcerative colitis.

In the qualitative immunoassay, the fecal or bodily sample is diluted 10 fold and added to a well containing immobilized neutrophilic antigens. If endogenous fecal ANCA is present, it will bind to the neutrophilic antigens during an incubation step at 37°C. Following the incubation, polyvalent antibodies to human immunoglobulin coupled to an enzyme, such as a horseradish peroxidase enzyme, (conjugate) is added and allowed to bind to captured ANCA. Unbound conjugate is then washed from the well and one component substrate (e.g., tetramethylbenzidene and hydrogen peroxide) is added for color development. Following the substrate incubation, 0.1M sulfuric acid is added to stop the reaction and the optical density (OD) is obtained spectrophotometrically at 450 nm.

In a clinical study, a total of 98 IBD patients were enrolled and comprised 51% males and 49% females with an age range of 0 to 69 years. The approximate 1 to 1 ratio is similar to the ratio observed in IBD patient populations. The IBS patient group had an age range of 5 to 39 years with 57% males and 43% females. The healthy controls were 55% male and 45% female and comprised the age range of 20 to 79 years. Individual numbers for each age group are shown in Table 1.

TABLE 1. Summary of patient population.

Summary of Clinical Histories (N=116)	Total Subjects
Total number of IBD patients	98
No. Males	50
No. Females	48
Total number of patients with Crohn's Disease	47
No. Males	26
No. Females	21
Total number of patients with ulcerative colitis	51
No. Males	24
No. Females	27
Total number of patients with irritable bowel syndrome	7
No. Males	4
No. Females	3
Total number of healthy persons	11
No. Males	6
No. Females	5

There were 51 ulcerative colitis (UC) patients, 47 Crohn's disease (CD) patients, 7 irritable bowel patients (IBS), and 11 healthy (H) adults recruited for the study. Fecal specimens were collected from each enrolled patient and stored at -70°C until tested. Specimen consistency ranged from solid to liquid. The level of fecal ANCA was determined using the qualitative ANCA ELISA as previously described. Disease activity was defined using elevated fecal lactoferrin as an indicator of intestinal inflammation. A dilution of 1:10 was used in the qualitative ELISA test and results were reported as positive (absorbance values ≥ 0.140) or negative (absorbance values < 0.140). The mean

optical densities, standard deviation and P values (two-tailed student T-test with unequal variance) were determined for the ANCA positive ulcerative colitis patients. Of the 26 patients that tested positive for fecal ANCA, there were four patients had Crohn's Disease, 21 had ulcerative colitis and one patient was healthy. ANCA-positive ulcerative colitis showed a mean ± SD OD_{450} of 0.311 ± 0.166. The mean optical density for the ulcerative colitis patients was significantly different from IBS and healthy persons (p value<0.0005). A summary of the statistical analysis is listed in Table 2.

TABLE 2. Summary of the mean, standard deviation and P values for qualitative ELSA test Optical Densities

Group	Number	Mean Optical Density	Standard Deviation	Optical Density Range	P values
ANCA + UC	21	0.311	0.166	0.141-0.804	UC vs CD p<0.5
ANCA + CD	4	0.209	0.115	0.141-0.381	UC vs CD, IBS, H p<0.0005
IBS	7	0.078	0.027	0.047-0.121	UC vs CD, IBS p<0.005
Healthy	11	0.071	0.041	0.039-0.104	UC vs IBS, H p<0.0005

In the group of patients with IBD, there were 47 with Crohn's disease and 51 with ulcerative colitis. In the ulcerative colitis group, 41% were positive. In the Crohn's disease group, a total of 9% patients were positive using the qualitative ELISA test. Of the 11 healthy persons, 1 was positive and all 7 IBS patients were negative by the qualitative ELISA test. A summary of positive results for the qualitative ELISA test are shown in Table 3 and individual results are listed in Table 4 and Table 5.

TABLE 3. Summary of positive results for Crohn's disease, ulcerative colitis, and IBS

Total Assessments N = 116	Total	Fecal ANCA Positive	Fecal ANCA Negative
Total IBD (Crohn's disease and ulcerative colitis)	98	26% (25)	75% (73)
Total Crohn's Disease	47	9% (4)	91% (43)
Total Ulcerative Colitis	51	41% (21)	59% (30)
Total IBS	7	0	7
Total Healthy Persons	11	9%(1)	91%(10)

When distinguishing ulcerative colitis from Crohn's disease, the qualitative ELISA test exhibited a sensitivity of 41% and specificity of 92%. The predictive positive and negative values were 84% and 59%, respectively, and the correlation was 65% (Table 4).

TABLE 4. Statistical evaluation using the qualitative ELISA test to distinguish Crohn's disease from ulcerative colitis

N=98	Ulcerative colitis	Crohn's disease
ANCA positive	21	4
ANCA negative	30	43

Sensitivity	41%
Specificity	92%
Predictive Positive Value	84%
Predictive Negative Value	59%
Correlation	65%

When distinguishing ulcerative colitis from irritable bowel syndrome and healthy persons, the qualitative ELISA test exhibited a sensitivity of 41% and a specificity of 92%. The predictive positive and negative values

were 81% and 67%, respectively, and the correlation was 70% as shown in Table 5.

TABLE 5. Statistical evaluation using the qualitative ELISA test to distinguish ulcerative colitis from Crohn's disease, irritable bowel syndrome and healthy persons

N=116	Ulcerative colitis	Crohn's disease IBS/Healthy
ANCA positive	21	5
ANCA negative	30	60

Sensitivity	41%
Specificity	92%
Predictive Positive Value	81%
Predictive Negative Value	67%
Correlation	70%

The sensitivity of the qualitative ELISA test was determined using serial two fold dilutions of human ANCA positive serum. For the analysis, standard curves were generated using the sample diluent. The test was consistently positive to a titer of 0.063 as determined by a cutoff absorbance value of ≥ 0.200. Individual results are shown below in Table 6 and standard curves are shown in FIG. 1.

TABLE 6. Standard curves generated using qualitative ELISA test (cut-offs are in bold)

Human ANCA Serum	Test 1	Test 2	Test 3	Mean	StDev
1.000 (Neat)	1.441	1.469	1.525	1.478	0.043
0.500	1.098	0.941	1.014	1.018	0.079
0.250	0.717	0.595	0.666	0.659	0.061
0.125	0.492	0.428	0.444	0.455	0.033
0.063	0.327	0.303	0.320	0.317	0.012
0.032	0.196	0.295	0.221	0.237	0.051
0.016	0.132	0.184	0.179	0.165	0.029
Diluent	0.067	0.093	0.109	0.090	0.021

Table 7, below, contains the clinical data and test results for patients with ulcerative colitis that participated in the study. Table 8, below, contains the clinical data and test results for patients with Crohn's disease that participated in the study. Table 9, below, contains the clinical data and test

results for patients with irritable bowel syndrome that participated in the study. Table 10, below, contains the clinical data and test results for health patients that participated in the study.

TABLE 7. Clinical data and ELISA results for ulcerative colitis patients.

Patient ID	Sex	Age Range	Disease	Disease Activity	OD	ELISA Result
UC1	F	10-19	UC	INACTIVE	0.053	NEGATIVE
UC2	F	5-9	UC	INACTIVE	0.107	NEGATIVE
UC3	F	5-9	UC	ACTIVE	0.058	NEGATIVE
UC4	M	10-19	UC	INACTIVE	0.048	NEGATIVE
UC5	M	10-19	UC	ACTIVE	0.512	POSITIVE
UC6	F	10-19	UC	ACTIVE	0.061	NEGATIVE
UC7	M	5-9	UC	ACTIVE	0.211	POSITIVE
UC8	M	10-19	UC	ACTIVE	0.106	NEGATIVE
UC9	M	10-19	UC	INACTIVE	0.804	POSITIVE
UC10	M	10-19	UC	ACTIVE	0.091	NEGATIVE
UC11	F	10-19	UC	ACTIVE	0.169	POSITIVE
UC12	F	10-19	UC	ACTIVE	0.209	POSITIVE
UC13	F	10-19	UC	ACTIVE	0.351	POSITIVE
UC14	F	10-19	UC	ACTIVE	0.198	POSITIVE
UC15	F	5-9	UC	ACTIVE	0.098	NEGATIVE
UC16	F	5-9	UC	ACTIVE	0.050	NEGATIVE
UC17	F	10-19	UC	ACTIVE	0.091	NEGATIVE
UC18	M	10-19	UC	ACTIVE	0.603	POSITIVE
UC19	M	10-19	UC	ACTIVE	0.091	NEGATIVE
UC20	F	10-19	UC	ACTIVE	0.142	POSITIVE
UC21	M	10-19	UC	ACTIVE	0.074	NEGATIVE
UC22	F	10-19	UC	ACTIVE	0.105	NEGATIVE
UC23	M	10-19	UC	INACTIVE	0.256	POSITIVE
UC24	F	0-4	UC	ACTIVE	0.308	POSITIVE
UC25	F	5-9	UC	ACTIVE	0.072	NEGATIVE
UC26	M	10-19	UC	INACTIVE	0.237	POSITIVE
UC27	M	10-19	UC	ACTIVE	0.048	NEGATIVE
UC28	M	10-19	UC	ACTIVE	0.049	NEGATIVE
UC29	M	10-19	UC	ACTIVE	0.059	NEGATIVE
UC30	F	10-19	UC	INACTIVE	0.047	NEGATIVE
UC31	M	10-19	UC	ACTIVE	0.055	NEGATIVE
UC32	M	10-19	UC	INACTIVE	0.044	NEGATIVE
UC33	F	10-19	UC	ACTIVE	0.043	NEGATIVE

UC34	M	5-9	UC	ACTIVE	0.046	NEGATIVE
UC35	M	10-18	UC	INACTIVE	0.043	NEGATIVE
UC36	M	10-17	UC	INACTIVE	0.040	NEGATIVE
UC37	F	10-19	UC	ACTIVE	0.047	NEGATIVE
UC38	F	0-4	UC	ACTIVE	0.049	NEGATIVE
UC39	F	5-9	UC	INACTIVE	0.363	POSITIVE
UC40	F	10-19	UC	INACTIVE	0.046	NEGATIVE
UC41	M	10-19	UC	ACTIVE	0.118	NEGATIVE
UC42	F	50-59	UC	ACTIVE	0.230	POSITIVE
UC43	M	10-19	UC	ACTIVE	0.051	NEGATIVE
UC44	F	30-39	UC	ACTIVE	0.060	NEGATIVE
UC45	F	50-59	UC	ACTIVE	0.465	POSITIVE
UC46	M	50-59	UC	ACTIVE	0.274	POSITIVE
UC47	F	30-39	UC	ACTIVE	0.141	POSITIVE
UC48	M	60-69	UC	ACTIVE	0.184	POSITIVE
UC49	F	40-49	UC	ACTIVE	0.397	POSITIVE
UC50	F	40-49	UC	ACTIVE	0.337	POSITIVE
UC51	M	30-39	UC	ACTIVE	0.143	POSITIVE

TABLE 8. Clinical data and ELISA results for Crohn's disease patients.

Patient ID	Sex	Age Range	Disease	Disease Activity	OD	Result
CD1	M	10-19	CD	ACTIVE	0.050	NEGATIVE
CD2	M	10-19	CD	ACTIVE	0.113	NEGATIVE
CD3	M	10-19	CD	ACTIVE	0.050	NEGATIVE
CD4	F	10-19	CD	ACTIVE	0.381	POSITIVE
CD5	F	10-19	CD	ACTIVE	0.058	NEGATIVE
CD6	M	10-19	CD	INACTIVE	0.068	NEGATIVE
CD7	M	10-19	CD	ACTIVE	0.066	NEGATIVE
CD8	M	5-9	CD	ACTIVE	0.059	NEGATIVE
CD9	F	10-19	CD	ACTIVE	0.059	NEGATIVE
CD10	F	10-19	CD	ACTIVE	0.065	NEGATIVE
CD11	F	10-19	CD	INACTIVE	0.055	NEGATIVE
CD12	M	10-19	CD	INACTIVE	0.071	NEGATIVE
CD13	F	10-19	CD	ACTIVE	0.065	NEGATIVE
CD14	M	10-19	CD	ACTIVE	0.098	NEGATIVE
CD15	F	10-19	CD	ACTIVE	0.099	NEGATIVE
CD16	M	10-19	CD	ACTIVE	0.166	POSITIVE
CD17	F	10-19	CD	ACTIVE	0.147	POSITIVE
CD18	M	10-19	CD	ACTIVE	0.057	NEGATIVE

CD19	F	10-19	CD	ACTIVE	0.084	NEGATIVE
CD20	M	10-19	CD	ACTIVE	0.053	NEGATIVE
CD21	F	10-19	CD	ACTIVE	0.074	NEGATIVE
CD22	M	10-19	CD	ACTIVE	0.054	NEGATIVE
CD23	M	0-5	CD	ACTIVE	0.055	NEGATIVE
CD24	M	10-19	CD	ACTIVE	0.067	NEGATIVE
CD25	M	10-19	CD	ACTIVE	0.099	NEGATIVE
CD26	M	5-9	CD	ACTIVE	0.086	NEGATIVE
CD27	F	10-19	CD	ACTIVE	0.043	NEGATIVE
CD28	F	10-19	CD	ACTIVE	0.064	NEGATIVE
CD29	M	5-9	CD	INACTIVE	0.039	NEGATIVE
CD30	M	10-19	CD	ACTIVE	0.071	NEGATIVE
CD31	F	10-15	CD	ACTIVE	0.109	NEGATIVE
CD32	M	10-19	CD	INACTIVE	0.057	NEGATIVE
CD33	M	10-19	CD	ACTIVE	0.141	POSITIVE
CD34	M	10-19	CD	INACTIVE	0.045	NEGATIVE
CD35	F	10-19	CD	ACTIVE	0.051	NEGATIVE
CD36	F	10-19	CD	ACTIVE	0.132	NEGATIVE
CD37	F	10-19	CD	INACTIVE	0.046	NEGATIVE
CD38	M	10-19	CD	ACTIVE	0.057	NEGATIVE
CD39	F	20-29	CD	INACTIVE	0.051	NEGATIVE
CD40	F	20-29	CD	ACTIVE	0.053	NEGATIVE
CD41	M	50-59	CD	ACTIVE	0.060	NEGATIVE
CD42	F	50-59	CD	ACTIVE	0.062	NEGATIVE
CD43	M	20-29	CD	ACTIVE	0.056	NEGATIVE
CD44	F	60-69	CD	ACTIVE	0.130	NEGATIVE
CD45	M	60-69	CD	ACTIVE	0.078	NEGATIVE
CD46	F	40-49	CD	ACTIVE	0.116	NEGATIVE
CD47	M	60-69	CD	ACTIVE	0.057	NEGATIVE

TABLE 9. Clinical data and ELISA results for Irritable bowel syndrome patients.

Patient ID	Sex	Age Range	Disease	ELISA OD#s	ELISA Results
IBS1	F	10-19	IBS	0.056	NEGATIVE
IBS2	M	10-19	IBS	0.047	NEGATIVE
IBS3	M	5-9	IBS	0.099	NEGATIVE
IBS4	M	10-19	IBS	0.068	NEGATIVE
IBS5	M	10-19	IBS	0.092	NEGATIVE
IBS6	F	20-29	IBS	0.121	NEGATIVE
IBS7	F	30-39	IBS	0.064	NEGATIVE

TABLE 10. Clinical data and ELISA results for healthy persons.

Subject ID	Sex	Age Range	ELISA OD#s	ELISA Results
D1	F	40-49	0.087	NEGATIVE
D2	M	20-29	0.078	NEGATIVE
D5	M	20-29	0.178	POSITIVE
D15	M	50-59	0.041	NEGATIVE
D17	M	50-59	0.039	NEGATIVE
D18	F	40-49	0.069	NEGATIVE
D19	F	60-69	0.050	NEGATIVE
D20	M	70-79	0.039	NEGATIVE
D21	F	70-79	0.104	NEGATIVE
D22	M	60-69	0.045	NEGATIVE
D24	F	50-59	0.054	NEGATIVE

In summary, the present invention is directed to non-invasive methods for aiding in the differentiation of ulcerative colitis from Crohn's disease by determining the presence of ANCA as a marker of ulcerative colitis. The present invention is further drawn to immunoassays, e.g., qualitative enzyme-linked immunoassays, that utilize antibodies specific to human immunoglobulins for the measurement of total endogenous ANCA in human feces. The present invention has been described in relation to particular embodiments which are intended in all respects to be illustrative rather than restrictive. Alternative embodiments will become apparent to those skilled in the art to which the present invention pertains without departing from its scope.

From the foregoing, it will be seen that this invention is one well adapted to attain all the ends and objects hereinabove set forth together with other advantages which are obvious and which are inherent to the method.

It will be understood that certain features and subcombinations are of utility and may be employed without reference to other features and subcombinations. This is contemplated by and is within the scope of the claims.

CLAIMS

Having thus described the invention, what is claimed is:

1. A method for testing a fecal sample, the method comprising: obtaining a fecal sample from a person; and determining whether anti-neutrophil cytoplasmic antibodies are present in the sample.

2. The method of claim 1, wherein if the sample contains anti-neutrophil cytoplasmic antibodies, a diagnosis of ulcerative colitis may be substantially concluded.

3. The method of claim 2, wherein the presence of anti-neutrophil cytoplasmic antibodies is used to aid in the differentiation of ulcerative colitis from Crohn's disease.

4. The method of claim 2, wherein the presence of anti-neutrophil cytoplasmic antibodies is used to aid in the differentiation of ulcerative colitis from other gastrointestinal illnesses.

5. The method of claim 4, wherein the other gastrointestinal illness is irritable bowel syndrome.

6. The method as recited in claim 1, wherein the endogenous anti-neutrophil cytoplasmic antibodies comprise the total anti-neutrophil cytoplasmic antibodies.

7. The method as recited in claim 1, further comprising: diluting the fecal sample.

8. The method as recited in claim 7, further comprising: contacting the sample with neutrophil cytoplasmic antigens to create a treated sample.

9. The method as recited in claim 8, further comprising: contacting the treated sample with polyvalent antibodies to human immunoglobulin to create a readable sample.

10. The method as recited in claim 9, further comprising: determining an optical density of the readable sample at 450 nm, wherein the optical density corresponds to a level of endogenous anti-neutrophil cytoplasmic antibodies in the sample.

11. A diagnostic assay for diagnosing ulcerative colitis by determining the endogenous anti-neutrophil cytoplasmic antibodies, the assay comprising: obtaining a human fecal sample; diluting the fecal sample; contacting the sample with neutrophil cytoplasmic antigens to create a treated sample; contacting the treated sample with polyvalent antibodies to human immunoglobulin to create a readable sample; determining the optical density of the readable sample at 450 nm.

12. The diagnostic assay as recited in claim 11, wherein if the readable sample contains endogenous anti-neutrophil cytoplasmic antibodies, a diagnosis of ulcerative colitis is substantially concluded.

13. The diagnostic assay as recited in claim 12, wherein the antibodies are one of IgG, IgE, IgM, IgD, IgA_{sec}, IgA, and combinations thereof.

14. The diagnostic assay as recited in claim 1, wherein the assay comprises one of an enzyme-linked immunoassay and a lateral flow membrane test.

15. A kit for diagnosing ulcerative colitis by testing a fecal sample from a person to be diagnosed, the kit comprising: one or more microassay plates, each the plate containing neutrophil cytoplasmic antigens; polyvalent antibodies to human immunoglobulin; and enzyme substrate for color development.

16. The kit as recited in claim 15, further comprising a stop solution for quenching the reaction.

17. A method for screening for ulcerative colitis, the method comprising: obtaining a sample from a person; determining whether anti-neutrophil cytoplasmic antibodies are present in the sample; and if so, a diagnosis of ulcerative colitis may be substantially concluded.

18. The method of claim 17, wherein the presence of anti-neutrophil cytoplasmic antibodies is used to aid in the differentiation of ulcerative colitis from Crohn's disease.

19. The method of claim 17, wherein the presence of anti-neutrophil cytoplasmic antibodies is used to aid in the differentiation of ulcerative colitis from other gastrointestinal illnesses.

20. The method as recited in claim 17, wherein the endogenous anti-neutrophil cytoplasmic antibodies comprise the total anti-neutrophil cytoplasmic antibodies.

21. The method as recited in claim 17, further comprising: diluting the sample.

22. The method as recited in claim 21, further comprising: contacting the sample with neutrophil cytoplasmic antigens to create a treated sample.

23. The method as recited in claim 22, further comprising: contacting the treated sample with polyvalent antibodies to human immunoglobulin to create a readable sample.

24. The method as recited in claim 23, further comprising: determining an optical density of the readable sample at 450 nm, wherein the optical density corresponds to a level of endogenous anti-neutrophil cytoplasmic antibodies in the sample.

25. The method as recited in claim 17, wherein the sample is one of human feces, whole blood, serum, plasma, human bodily fluid and human tissue.

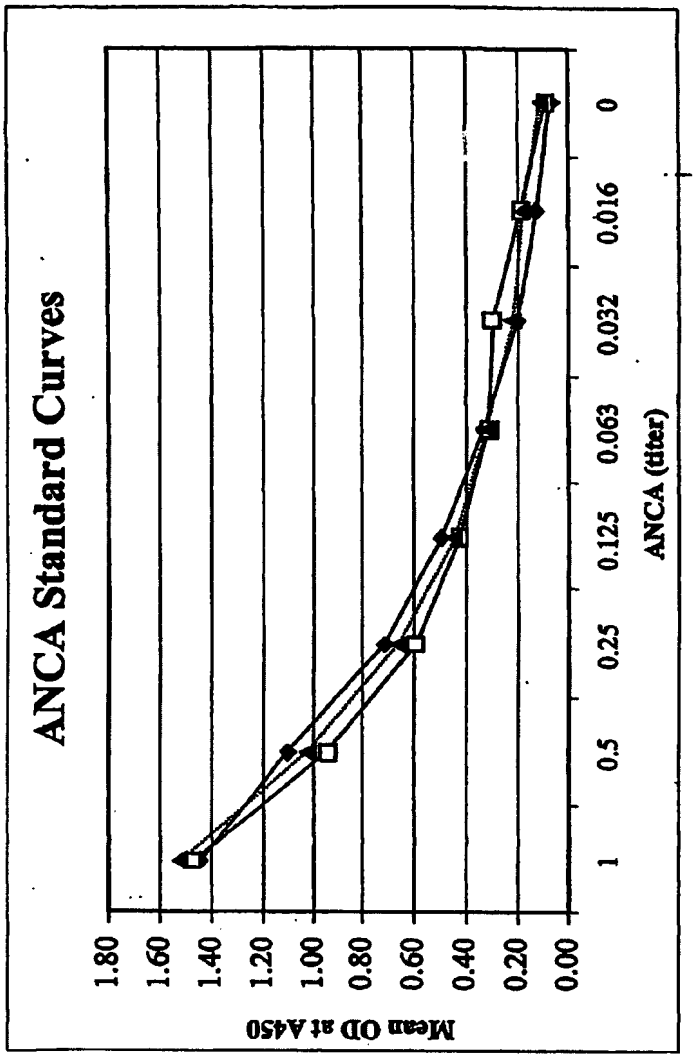

ANCA Standard Curves
Mean OD at A450
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1
0.5
0.25
0.125
0.063
0.032
0.016
0
ANCA (titer)

FIG. 1